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Review

A model international partnership for community-based research on vaccine-preventable diseases: The Kamphaeng Phet-AFRIMS Virology Research Unit (KAVRU)

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ABSTRACT

This paper describes an international collaboration to carry out studies that contributed to the understanding of pathogenesis, diagnosis, treatment, and prevention of several diseases of public health importance for Thailand and the United States. In Kamphaeng Phet Province, Thailand, febrile syndromes, including encephalitis, hepatitis, hemorrhagic fever, and influenza-like illnesses, occurred commonly and were clinically diagnosed, but the etiology was rarely confirmed. Since 1982, the Kamphaeng Phet Provincial Hospital, the Thai Ministry of Public Health, and the US Army Component of the Armed Forces Research Institute of Medical Sciences, along with vaccine manufacturers and universities, have collaborated on studies that evaluated and capitalized on improved diagnostic capabilities for infections caused by Japanese encephalitis, hepatitis A, dengue, and influenza viruses. The collaboration clarified clinical and epidemiological features of these infections and, in large clinical trials, demonstrated that vaccines against Japanese encephalitis and hepatitis A viruses were over 90% efficacious, supporting licensure of both vaccines. With the introduction of Japanese encephalitis vaccines in Thailand's Expanded Program on Immunization, reported encephalitis rates dropped substantially. Similarly, in the US, particularly in the military populations, rates of hepatitis A disease have dropped with the use of hepatitis A vaccine. Studies of the pathogenesis of dengue infections have increased understanding of the role of cellular immunity in responding to these infections, and epidemiological studies have prepared the province for studies of dengue vaccines. Approximately 80 publications resulted from this collaboration. Studies conducted in Kamphaeng Phet provided experience that contributed to clinical trials of hepatitis E and HIV vaccines, conducted elsewhere. To provide a base for continuing studies, The Kamphaeng Phet-AFRIMS Virology Research Unit (KAVRU) was established. This paper reviews the origins of the collaboration and the scientific observations made between 1982 and 2012.

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Contents

1. Introduction

Vaccine efficacy trials are best conducted in areas where the incidence and epidemiology of the disease of interest is well-documented and recognized as important by local public health, medical and political leaders, as well as the community at-large. Of additional importance are the willingness of subjects to participate, their access to medical care and laboratory diagnostic facilities, and the clinical trials infrastructure supporting the various aspects of trial design and execution.

Less tangible are mutual respect, trust and long-term commitment between investigators, and these factors are particularly important for studies conducted jointly by host-country and visiting foreign investigators in which professional relationships are as important as formal institutional agreements.

We review a successful collaboration between investigators at Kamphaeng Phet Province (KPP) Hospital, Thailand, the Ministry of Public Health (MOPH) of Thailand, the US Army Medical Component-Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok, and other organizations. The collaboration took place in Kamphaeng Phet Province (KPP) in northern Thailand. The province was defined as a political entity before 1000 AD, and ruins from this period have been declared a UNESCO World Heritage Site. The province is generally hilly, though flat portions support the cultivation of rice, bananas, sugar cane, and tapioca, irrigated by the Ping River. The province covers 8600 km^2 subdivided into 11 districts containing just over 800 villages; the 2011 population was 726,000. Each village has an elected mayor or village head. Health care is provided hierarchically from the provincial hospital to the district hospitals to the village-based public health offices.

Nearly all sub-districts (tumbons) have a government school. Like the political and health care system, the educational system is organized hierarchically from the center of the province to the districts and to sub-districts and villages. Periodic meetings of school administrators are held to disseminate important information. The structure and efficiency of the school system is conducive to the study of diseases that primarily afflict school-aged children.

Partners in the collaboration contributed from their strengths, and the convergence of capabilities facilitated the many successful investigations that were carried out. KPP contributed medical facilities, understanding of the impact and management of local disease problems and their management, public health infrastructure, schools, and prospective volunteers. The Thai MOPH brought expertise in disease surveillance and outbreak control and perspective on disease priorities. Investigators from the US Army contributed advances in diagnosis, knowledge of vaccines, entomological support, and strategies for study design. Vaccine manufacturers provided vaccines. Investigators at universities in Thailand and the US contributed their skills as well.

This collaboration led to studies that contributed to regulatory approval of two vaccines (Japanese encephalitis and hepatitis A vaccines) in two countries (Thailand and the US), knowledge of the pathogenesis and epidemiology of dengue, and understanding of the transmission of influenza. The collaboration also provided models for two major vaccine (hepatitis E and human

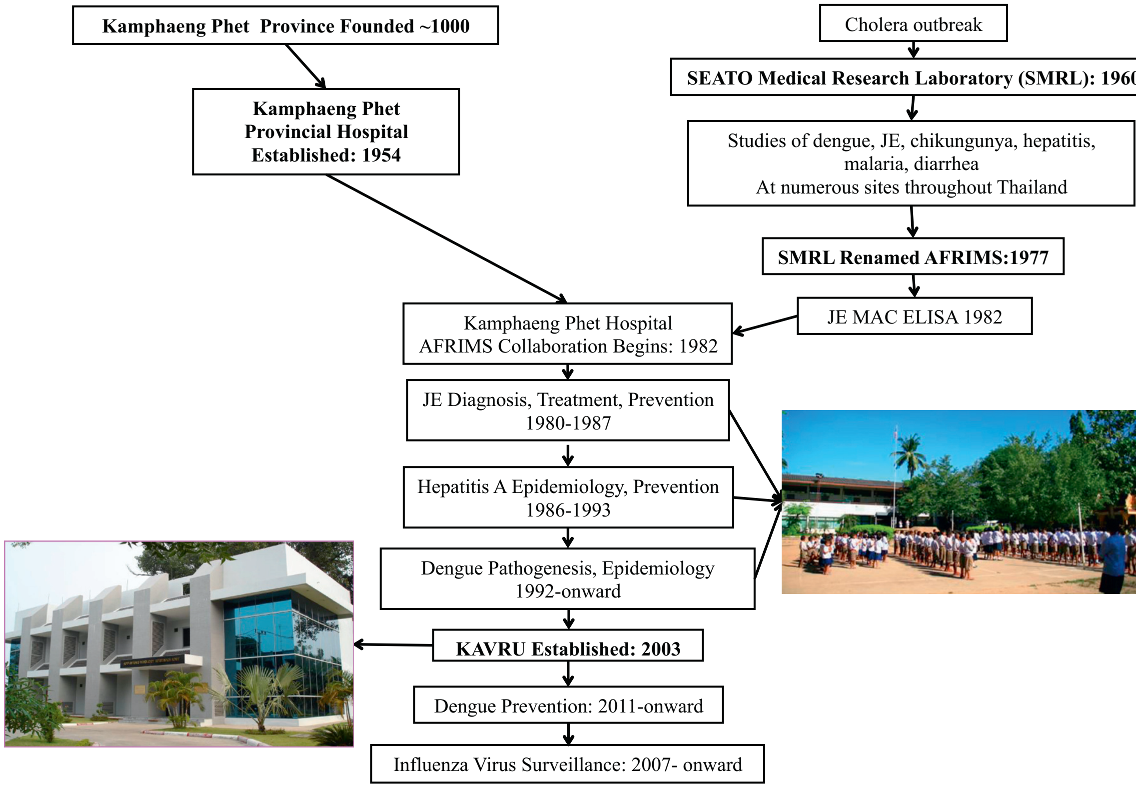

Fig. 1. Overview of collaborative studies performed in Kamphaeng Phet Province, Thailand.

immunodeficiency virus vaccines) trials conducted elsewhere. This paper reviews scientific progress derived from approximately 80 published studies carried out in KPP, emphasizing the personal and professional interactions that sustain such collaborations.

2. History of the collaboration

2.1. KPP Hospital

KPP Hospital was built in 1954 and expanded several times to the current 410 beds. The hospital cares for approximately 400,000 outpatients and 40,000 inpatients per year and has a modern laboratory and radiology department. Discharge records include listings of final diagnoses for all admitted patients. Because the hospital is the principal focus for medical care in the province, it is well suited to serve as the headquarters for population-based studies.

2.2. Thai Ministry of Public Health

In addition to many other missions, the Thai Ministry of Public Health conducted communicable disease surveillance that allowed public health leaders to identify a number of diseases, including Japanese encephalitis and dengue hemorrhagic fever, as substantial country-wide problems [2–5].

2.3. South East Asia Treaty Organization (SEATO) Medical Research Laboratory (SMRL), Bangkok, Thailand (Fig. 1) [1]

In 1958–59, in response to a cholera epidemic in Thailand, the Thai Government invited the US to join Thai investigators in understanding and managing the epidemic. A delegation, representing the US Navy, Army, and Public Health Service, visited Thailand's Undersecretary of State for Health, who had been a medical school roommate of a member of the US delegation. The King of Thailand emphasized that the international research to be conducted by the new laboratory should benefit his subjects. The South East Asia Treaty Organization (SEATO) Thai Cholera Research Laboratory was thereby established, with the Undersecretary appointed the first Director General. In 1960, the Cholera Laboratory was renamed the SEATO Medical Research Laboratory (SMRL), with Thai and US components (Fig. 1).

2.4. The Armed Forces Research Institute of Medical Sciences (AFRIMS)

In 1977, SEATO was disbanded due to waning political interest in the overall organization, and the SMRL was renamed the Armed Forces Research Institute of Medical Sciences (AFRIMS), which is led by a Thai Military Director General. The U.S. Army component is commanded by a US Army Officer who reports to the Walter Reed Army Institute of Research (WRAIR) Commander. When US Component studies involve human subjects, the study protocols must be approved by both the Thai and US Army Institutional Review Boards.

In the years since the formation of AFRIMS, and prior to the collaboration described in this paper, AFRIMS scientists and Thai colleagues had made notable contributions in several areas of mutual interest, including dengue (pathogenesis [6], neutralizing antibody [7,8], epidemiology [9], and surveillance [10]), chikungunya [11], Japanese encephalitis in northern Thailand [12], malaria treatment [13], vectors of dengue, malaria, and Japanese encephalitis [14], and hepatitis B antigenic subtypes [15]. These studies had been conducted in a number of sites throughout Thailand, and with the experience gained from these prior collaborations, coupled with surveillance of infectious disease health threats by scientists in the Thai Ministry of Public Health, teams from AFRIMS and the Thai Ministry of Public Health were well-positioned to establish a new long term study site in Kamphaeng Phet, at which site methods for control of some of these problems could be explored collaboratively.

2.5. Kamphaeng Phet-AFRIMS Virology Research Unit (KAVRU)

In 1982, AFRIMS scientists, seeking clinical specimens to evaluate a newly developed JE IgM antibody capture immunoassay (MAC ELISA) became aware that each summer the KPP Hospital admitted numerous pediatric patients with encephalitis. The hospital pediatric discharge diagnosis logbook revealed that the hospital provided care for many patients with encephalitis, as well as to patients diagnosed as having hemorrhagic fever, hepatitis, diarrhea, and pneumonia, suggesting a blueprint for multiple collaborative studies in the future.

As these studies unfolded, the hospital provided laboratory facilities and office space while AFRIMS provided technical support for virological diagnosis. The JE MAC ELISA evaluation was the first of many studies that led AFRIMS staff to become integrated with the community (doctors, nurses, school administrators, teachers, and parents) in the joint pursuit of public health research.

By 2001 the virology research laboratory was recognized as a valuable addition to the hospital's capabilities. Expansion was needed. Land was offered on the campus and funds for a new laboratory were obtained. The new laboratory was built and opened in 2003 as the KPP-AFRIMS Virology Research Unit, or KAVRU. The opening of KAVRU was co-officiated by the Permanent Secretary of the Thai Ministry of Public Health and the U.S. Ambassador to Thailand, as a joint Thailand-U.S. laboratory for the study of dengue and other emerging diseases. KAVRU was constructed as a state-of-the-art facility with its first floor dedicated to the clinical evaluation of subjects and processing/storage of specimens and the second floor to laboratory evaluation (including polymerase chain reaction testing). In light of increasing international requirements for vaccine trials, greater resources were applied to preparing the KAVRU study teams to comply with the latest regulatory standards.

3. Scientific results of the AFRIMS-KPP collaboration (Table 1)

3.1. Japanese encephalitis (Fig. 2)

In past years, AFRIMS investigators had investigated the ecology and epidemiology of JE in Chiang Mai province, and these studies laid the foundation for more sustained efforts in Kamphaeng Phet [21]. MOPH investigators conducted increasingly detailed surveillance of encephalitis [2] throughout Thailand, providing the MOPH of detailed knowledge of provinces with greatest risk (Table 1 and Fig. 2).

3.1.1. JE IgM antibody capture ELISA

The JE MAC ELISA test [16,17,19] that catalyzed the collaboration also revolutionized the diagnosis of arboviral encephalitis. Previously, etiological diagnosis had required a labor-intensive assay, performed on acute and convalescent sera, which provided a result long after discharge [21]. The new test could be performed quickly on small volumes of serum or CSF. The assay was used to establish the kinetics of antibody formation [18] and evaluate factors associated with fatal outcome [20]. The lead investigator shared data obtained in KPP with the US Centers for Disease Control Division of Vector Borne Diseases in Fort Collins, Colorado. AFRIMS offered training programs on performing the assay, eventually training scientists from many countries and serving as a reference laboratory as kits became commercialized [22–25]. The assay also facilitated performance of therapeutic studies. For example, dexamethasone had been used in treating JEV encephalitis patients, but a placebo-controlled study revealed no improvement in case fatality ratios in such patients [26].

Table 1
Results of studies of Japanese encephalitis, hepatitis A, dengue, and influenza conducted in Kamphaeng Phet Province.

JE diagnostic test (14 papers published)
Anti-JE IgM antibody detected in cerebrospinal fluid [16] and serum [17] by day 3 [18]
Many CSF specimens could be processed, facilitating disease surveillance [19]
Fatal outcomes were correlated with CSF virus, low antibody levels, and coma [20]
AFRIMS serves as reference laboratory for JE diagnostic kits [22–25]
Use of dexamethasone in treatment of acute JE does not increase survival [26]
JE vaccine (1 paper published)
JE vaccine was safe in Thai children with an efficacy of 91% [27]
The placebo cohort provided a prospective estimate of JE incidence [31]
Following an additional study [28], US FDA approved BIKEN JE vaccine
JE vaccine in Thailand introduced as routine immunization in Thailand, reducing rates
In the US, a cell culture JE vaccine, invented at WRAIR, licensed on basis of immunogenicity non-inferiority to vaccine tested in KPP [33,34]
KPP experience contributed to recommendations for global control of JE [35,36]
Hepatitis A (5 papers published)
Enrolled children from KPP: 65,000 for surveillance, 40,000 for vaccine trial
Hepatitis A incidence in KPP estimated to be about 11/100/year [47]
Hepatitis A vaccine was safe and 94% efficacious [49]
Licensure of the vaccine in the US was based in part on KPP trial results [50]
HAV vaccine was judged as not cost effective in Thailand [51]
Following use of HAV vaccine in the US Military, the hepatitis A incidence fell [53]
Dengue (56 papers published)
In JE vaccine study, DHF affected 107/100,000 and dengue fever, 135/100,000 [27]
Dengue and JE MAC ELISA calibrated to distinguish between dengue and JE [32]
Severity was correlated with higher viremia, antibody response, and DEN 2 [56,57]
Certain HLA types are at risk of more severe illness [58]
Rare neurological manifestations of dengue were identified [107]
Antibody titers may not be good markers of immunity [86]
The focal nature of dengue transmission was illuminated [80,93,103,113]
Site was prepared for phases 2 and 3 studies of dengue vaccines in rural Thai setting
17,815 children were enrolled in various studies
Phase 3 trial of dengue vaccine is ongoing
Influenza (4 papers published)
Participated in global influenza surveillance efforts [116–119]
An 800 person cohort was established for monitoring exposure to influenza viruses
Avian influenza associated with gender, smoking, and absent indoor plumbing [117]

3.1.2. JE vaccine in KPP

In 1982, investigators at AFRIMS asked the Director of the Thai Center for Disease Control about possible collaborative work. The Director presented surveillance data that revealed sharp annual outbreaks of encephalitis in northern provinces, making these outbreaks possibly amenable to control through the use of a vaccine if one was available [2]. JEV vaccines had been used in Japan, but they had not been shown to be efficacious in Thailand. The president of a company that made JE vaccine who was also associated with the Japan International Cooperation Agency (JICA) agreed that his company would provide vaccine and placebo for an efficacy trial. The head of the Thai National Institutes of Health had long felt that an efficacy trial of a JE vaccine in Thailand was needed, and she offered

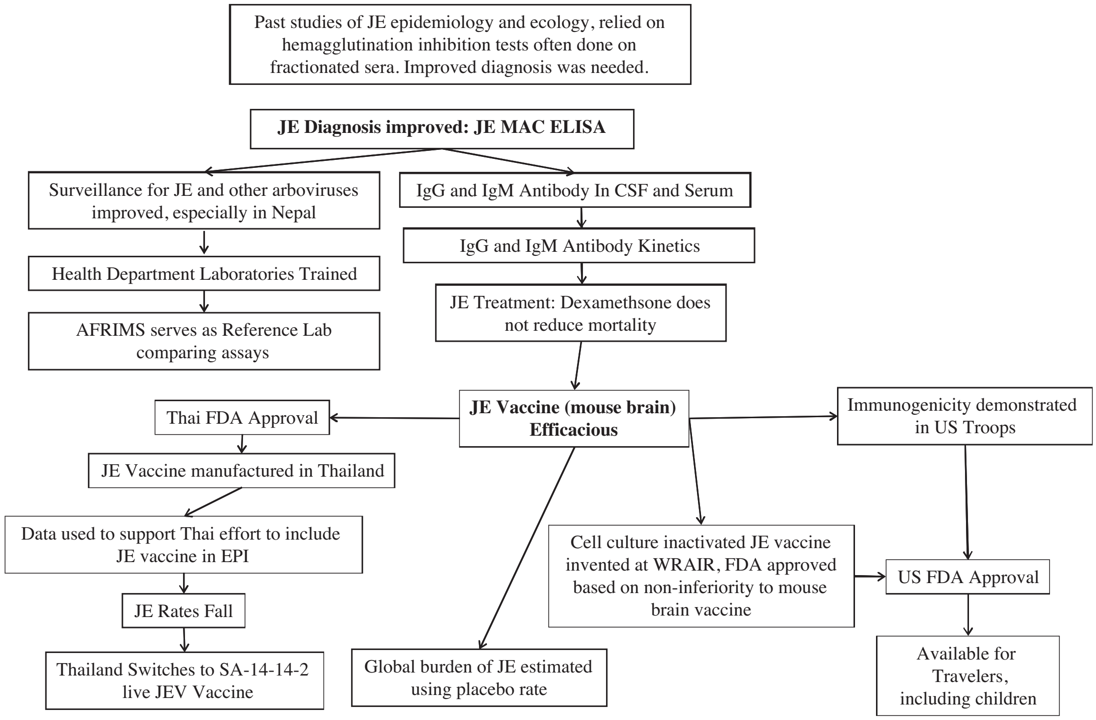

Fig. 2. Japanese encephalitis studies performed in Kamphaeng Phet Province by Thai Ministry of Public Health and US Army and the impact of those studies on Japanese encephalitis in Thai children and in US travelers.

her support. The provincial hospital director in KPP confirmed his support of an efficacy study.

Discussions between AFRIMS representatives and the Deputy Prime Minister of Thailand, members of Parliament, the Ministers of Health and Education, and the provincial governor, village heads, directors of primary and secondary schools, teachers, and parents provided a means by which all could be informed by the investigators. A protocol for the trial had been drafted, and the protocol was reviewed and approved by the Human Subjects Research Review Board of the US Army Surgeon General and the Thai MOPH Institutional Review Board, which in turn appointed a Thai oversight board to monitor the trial.

Between December 1984 and April 1985, more than 60,000 children from 496 villages in KPP were enrolled with parental consent and were vaccinated with one of two inactivated JE vaccines or tetanus toxoid [27]. The JE vaccine was made of virus purified from the brains of mice that had been inoculated with JE virus and inactivated with formalin. Enrollment and initial vaccination was accomplished by six teams, each team visiting three villages per day, followed by a second visit a week later to give a second dose. Each of the teams was led by a physician, and the MOPH provided many of these physicians from the Field Epidemiology Training Program conducted by the US Centers for Disease Control in Thailand. AFRIMS and KPP Hospital provided three nurses per team.

The JE vaccine efficacy was 91% [27], and these data contributed to Thai and US FDA licensure. The US FDA required one additional study to confirm safety and immunogenicity [28]. The Japanese company partnered with a company in the US to sponsor a New Drug Application and distribute vaccine. In Thailand the company facilitated construction of JE vaccine manufacturing facility. JE vaccine was phased into the Expanded Program on Immunization (EPI) throughout Thailand, resulting in a substantial decline in reported encephalitis cases [29,30]. Later, data from the placebo group provided rates of JE, contributing to global estimates of the burden of JEV [31].

In Thailand, a live attenuated JE vaccine has recently replaced the inactivated mouse brain vaccine, while, in the US, an inactivated JE vaccine produced in cell culture was invented at WRAIR as a replacement for the mouse brain vaccine tested in KPP, developed, under license to the Army, by a commercial manufacturer, and approved by the US FDA based on demonstration that the new vaccine was not inferior, with respect to stimulation of neutralizing antibody, to the vaccine that had been tested in KPP [33,34]. In addition, expertise gained in KPP contributed to recommendations for global control of JE [35,36].

Other studies demonstrated the presence of JE antigen in neurons in fatal cases [37], that non-immune pigs could be effective sentinels for JE virus circulation [38], that JE antibodies were detected in cells, blood and cerebrospinal fluid [39], and that JEV disease was more severe in patients in whom encephalitis preceded an immune response [42].

3.1.3. *Entomological studies*

Entomological studies revealed that mosquito cells derived from *Aedes pseudoscutellaris* were useful for bedside inoculation of specimens: serum and CSF from non-fatal JE cases failed to yield virus, while virus could be cultured from CSF from fatal cases or fresh brain collected at autopsy [40]. Isolations of virus from pooled collections of mosquitoes revealed that JE virus could be isolated for only a 10-day period around the time when the peak incidence of disease was observed in humans [41], suggesting that natural factors supporting virus transmission were transient.

3.2. *Hepatitis A studies (Fig. 3)*

3.2.1. *Invention of hepatitis A vaccine*

During the time in which the trial of JE vaccine was conducted in KPP, scientists at WRAIR had invented a test for detecting neutralizing antibody against hepatitis A [43], invented the world's first inactivated hepatitis A vaccine (HAV) [44], and protected monkeys against challenge. A clinical trial showed that the vaccine

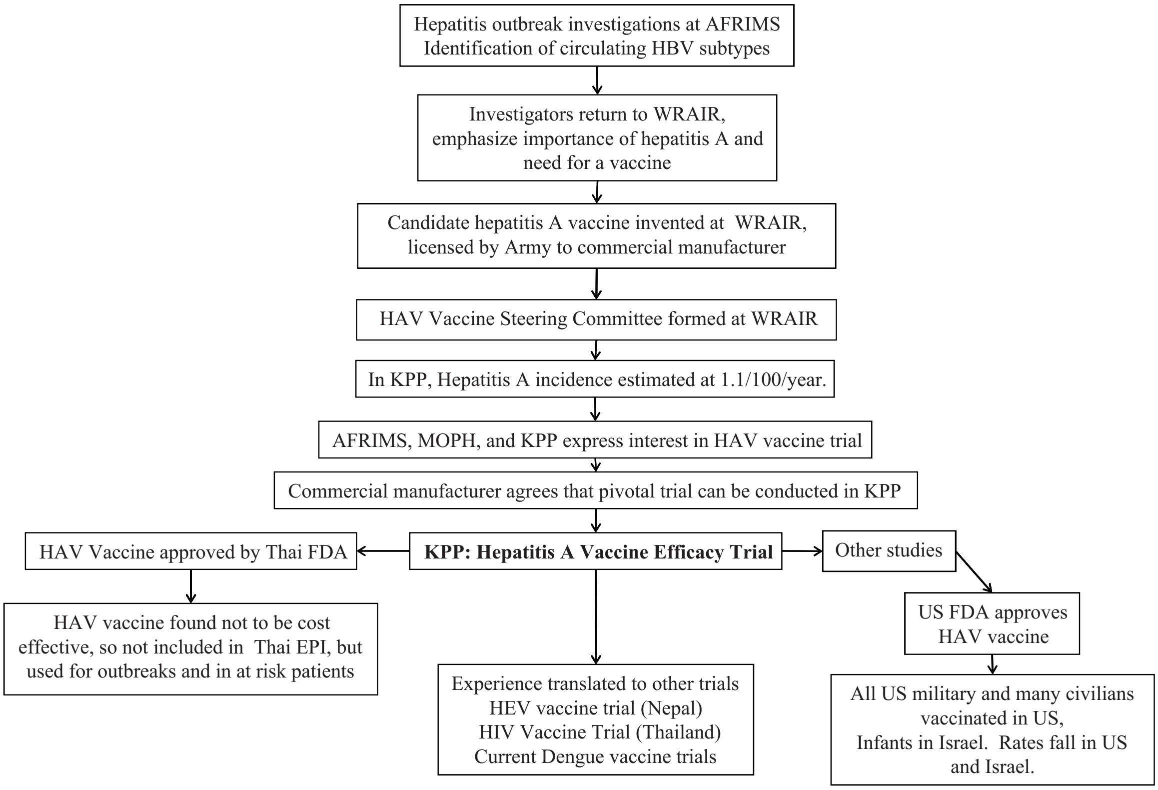

Fig. 3. Hepatitis A studies performed in US and Thailand, particularly in Kamphaeng Phet Province, leading to approved HAV vaccine.

stimulated neutralizing antibody in all eight volunteer recipients [45]. Using a "No Dollar Agreement", the Army transferred the HAV vaccine technology to a commercial vaccine manufacturer who in turn became the regulatory sponsor of the vaccine [46] (Fig. 3).

3.2.2. Hepatitis A vaccine trial in KPP

The manufacturer initially favored an efficacy trial in the US, but negotiations for such trials were unsuccessful, while epidemiological studies in KPP suggested that rates of hepatitis A (1.1 cases/100 children/year) [47] were substantial. Investigators at AFRIMS, along with Thai medical and public health leaders, felt that a collaborative HAV vaccine efficacy trial, similar to the recent JE vaccine efficacy trial, but with improvements in design, could be conducted in KPP. Eventually the company was convinced, and a collaborative team was assembled to conduct the second large-scale vaccine trial in Thailand. Approximately 42,000 children were enrolled to receive either hepatitis A or B vaccine, followed eventually by a crossover immunization. Surveillance for hepatitis A cases in the study population was conducted using a case definition of missed school, liver enzymes elevated above the upper limit of normal, and a positive test for hepatitis A IgM. Although not initially a part of the case definition, the US FDA requested that cases be confirmed by RT-PCR detection of viral RNA in feces, and, following substantial effort, data from this testing was added to the license application. Study participants ultimately received both hepatitis A and B vaccines and were provided medical care, and communities benefited by mitigation of hepatitis A outbreaks through construction of wells and renovation of toilet and hand washing facilities in schools.

The hepatitis A vaccine was found to be safe and 94% efficacious [48,49]. Publication of the results with Thai and US Army authors attested to the breadth of the collaboration. The vaccine was licensed in the US and Thailand based on data from KPP [50] and other studies, several of which were performed by Army investigators in military populations [46].

3.2.3. Post trial impacts

Analyses suggested that universal childhood immunization against HAV would not be cost effective in Thailand [51], so the vaccine was not incorporated into the routine immunization program. However, the HAV vaccine is used in Thailand for control of outbreaks, post exposure prophylaxis, and protection of persons at high risk of exposures. Lack of universal use of the HAV in Thailand provided the opportunity to discuss the ethics of conducting a trial after which vaccine was made available to populations other than those that participated in the trial [52].

In the United States, use of HAV vaccine was associated with a dramatic reduction of numbers of cases, especially in the military [53]. An even more precipitous decline in cases in all ages was observed in Israel following widespread immunization of toddlers [54].

3.3. Dengue

AFRIMS investigators and Thai colleagues had contributed to the understanding of the pathogenesis of dengue hemorrhagic fever [6]. Studies had relied on traditional diagnostic tests, including hemagglutination inhibition and neutralizing antibody assays [7,8] and various means of isolating dengue virus from clinical specimens, including mosquito inoculation and testing of tissues with immunofluorescence. A major advance had occurred with the discovery at WRAIR and distribution by the US CDC of mouse monoclonal antibodies specific for serotypes of dengue and other flaviviruses [56–58]. This monoclonal antibody technology was adapted rapidly at AFRIMS and expanded. Thai clinicians, particularly at Bangkok Children's Hospital, were recognized as experts in the diagnosis and clinical management of dengue hemorrhagic

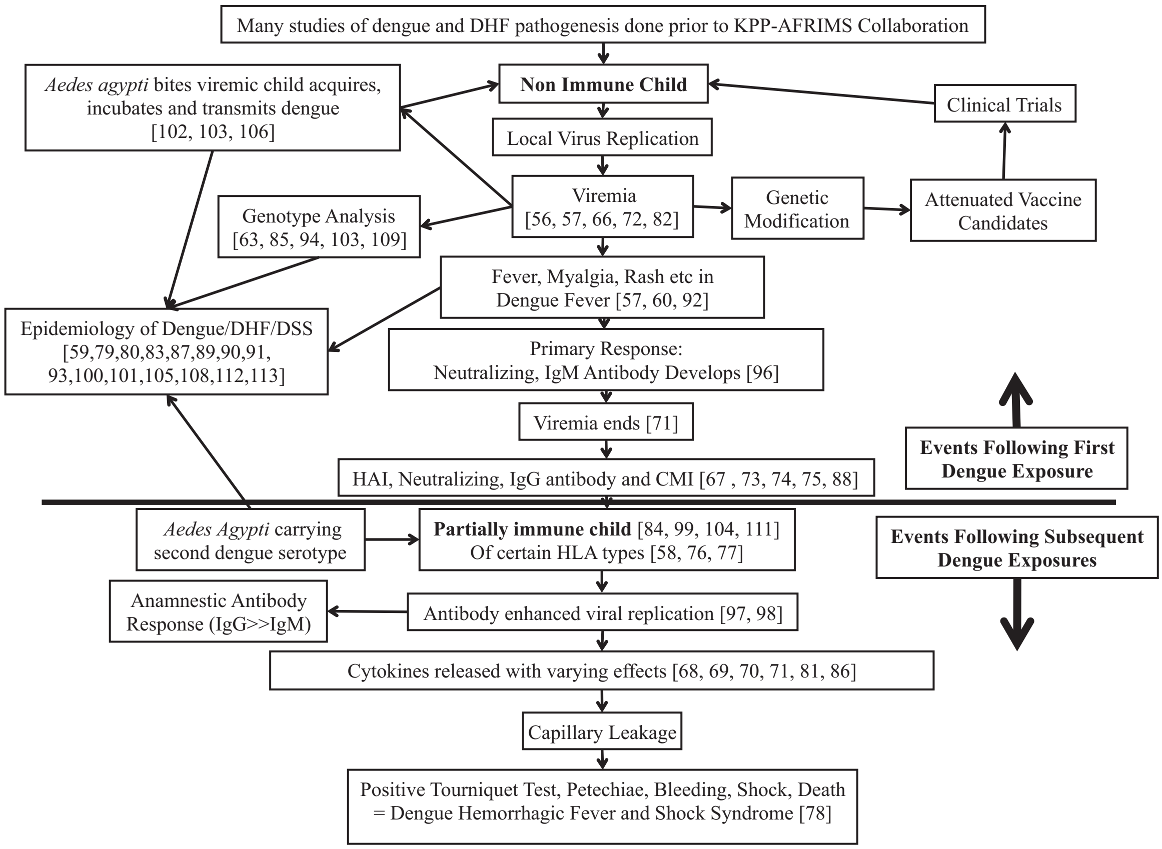

Fig. 4. Recent studies of dengue transmission, pathogenesis, diagnosis, and genetics conducted in Kamphaeng Phet Province.

fever, with diagnostic support provided by nearby AFRIMS. With the advent of improved techniques for detection of cytokine mediators in the immune response to dengue, investigators were in excellent position to conduct detailed studies at a fixed facility in KPP.

3.3.1. Dengue diagnosis

For many years serological diagnosis of dengue had relied on the hemagglutination inhibition test, which was challenging because of the great amount of cross-reactive antibody detected in this assay following any flavivirus infection. Surveillance in the JE vaccine efficacy trial revealed that dengue hemorrhagic fever hospitalizations occurred frequently, indicating that dengue was a significant problem for the children of KPP, providing an opportunity to refine the ELISA test to distinguish JE and dengue infections serologically in an area where both circulate [32] and suggesting that dengue vaccines might be evaluated in future studies. Analysis of specimens collected in KPP allowed refinement of the MAC ELISA assay used for JEV infections to diagnose JEV and dengue virus infections in an area where both viruses co-circulate [27].

3.3.2. Dengue pathogenesis (Fig. 4)

Dengue virus is a major cause of morbidity in Thailand, and Thai physicians have been leaders in understanding dengue pathophysiology, classification of cases, clinical management, and surveillance [55]. AFRIMS supported presentations in the diagnosis and management of dengue by leading Thai authorities to the staff at KPP Hospital (Fig. 4).

In 1994, investigators from the University of Massachusetts, Yale Arbovirus Research Laboratory in the US and, in Thailand, Siriraj Medical Molecular Biology Center, the Siriraj Hospital Institute of Pathology and Department of Transfusion Medicine, and the MOPH, began participation in studies that spanned 15 years, conducted largely at KPP Hospital, to better define the pathophysiology of dengue disease, provide insights into methods of prevention and treatment, and prospectively evaluate dengue virus transmission and disease in primary school children. More severe dengue was associated with higher viremia titers, secondary immune response, and dengue type 2 infection [56,57], and certain human leukocyte antigen types appeared to be at risk of more severe disease [58]. The burden of dengue disease appeared to be greater than estimated from surveillance data [59]. Additional studies have yielded a great deal of information about dengue in Thailand [60–113].

3.3.3. Dengue vaccine trials

Several dengue vaccine candidates were under development in different laboratories. By 2010, the manufacturer of a candidate dengue vaccine began preparations for a global multi-country Phase 3 trial, and KPP was identified as one of two sites in Thailand to be included. In collaboration with KPP Provincial Hospital, the Thai MOPH, Faculty of Tropical Medicine Mahidol University, the US Army, and the manufacturer, a Phase 3 dengue vaccine trial was initiated in Thailand in 2011. The manufacturer adopted surveillance methods developed in KPP for application to the entire phase 3 program. Vaccinations in the trial were completed in 2012 and active surveillance for dengue virus infections is ongoing.

3.3.4. Dengue vectors

Standardization of container descriptions and mosquito collection methods may facilitate evaluations of control strategies [90,108]. Dengue virus adaption to *Aedes agypti* populations depends on both virus and vector genotype [95]. Studies of breeding sites of *Aedes agypti* may lead to improved methods of vector control programs [100], as may observations that some households contribute disproportionately to dengue transmission [103], although residents may take measures against vectors mainly in response to the perceived nuisance of mosquitos [89].

3.4. Respiratory and other disease studies

Samples collected in the dengue studies were used in understanding the presence of other pathogens, including leptospirosis and metapneumovirus [110,114,115].

Prospective respiratory disease surveillance began in 2007, and KAVRU participated as a global influenza surveillance site [116–119]. With cases of H5N1 detected in both birds and humans in KPP, the need for influenza testing as a service to the province became apparent. In 2008, KAVRU began testing samples from the hospital and established PCR testing of respiratory samples on site. Subsequently, a cohort was enrolled to better understand human exposure to zoonotic influenza in KPP which suggested that exposure to avian influenza virus might be more common than previously thought [117].

4. Discussion

The establishment of a study site to evaluate a diagnostic test for JEV infection led, over 30 years, to studies supporting licensure of two vaccines, substantial scientific accomplishments, surveillance of etiologically confirmed disease, and pivotal experiences for scores of Thai and US researchers.

4.1. Site selection

Initially, the collaboration resulted from a simple site selection effort, guided by the documented presence of diseases of mutual interest and by personal relationships. Established Thai scientists at AFRIMS provided an essential network of expertise and trust among Thai governmental authorities. Established on a foundation of mutual interests, the collaboration grew over the years and included the construction of a modern laboratory (KAVRU) to provide laboratory support to ongoing studies of endemic and emerging diseases.

4.2. Beneficial joint collaboration

A hallmark of this collaboration has been the sensitive interaction by all participants to assure that studies addressed the expectations of sponsoring agencies. The Thai MOPH guided the collaboration toward the needs of the Thai people, as had been requested during the founding of the SMRL. Medical and educational authorities in KPP retained the focus on diagnosis, treatment and prevention of diseases affecting the people. AFRIMS investigators assured that studies addressed the needs of the US Army, which furnished resources programmed to find means to protect military personnel. Vaccine manufacturers contributed vaccine technology. Authorship on publications resulting from this collaboration reflects the participation of these agencies.

4.2.1. Shared experience

Both the Thai and US investigators grew through the shared experience derived from the many studies conducted in KPP. AFRIMS Department of Virology leadership rotated every 4–8 years, providing new perspectives, a link to technology under development at WRAIR, and experience with study design. The senior Thai investigators in the department provided continuity, expertise in diagnostic assays, and, most important, an interface with the Thai medical establishment. KPP hospital directors provided stability as well and participated in selection of studies and ongoing problem solving. Thai physicians and nurses possessed significant expertise in the treatment of these diseases they encountered on a daily basis. Thai MOPH leaders conducted careful disease surveillance and were well aware of epidemiological problems and interventions in need of study. Pooling of these talents resulted in research teams able to approach large public health problems.

A highly purified JEV vaccine, developed initially in the US during World War II to protect American Servicemen [120] and in Japan following World War II for the benefit of Japanese children, was evaluated in KPP, and the results of this evaluation led to the introduction and manufacturing of this vaccine in Thailand for the benefit of Thai children and to the licensure by the US FDA of the vaccine for the benefit of military personnel and travelers to JE endemic areas, completing an interrupted 40 year long process.

The HAV vaccine, invented at WRAIR, was also shown to be efficacious in KPP, providing additional experience for US and Thai investigators. A dengue vaccine trial is underway, and testing of additional dengue vaccines candidates is expected.

4.2.2. Applicability of scientific results in KPP to development of vaccines against a range of tropical infectious diseases

Studies in KPP that established the efficacy of the mouse brain derived JEV vaccine and of the HAV vaccine had direct bearing on more recent efforts to develop vaccines against these diseases. For example, now at WRAIR, the leader of the HAV vaccine trial assembled a group that invented an inactivated cell culture JEV vaccine produced in vero cells that became licensed in the US based on demonstration of serological non-inferiority to the mouse brain vaccine tested in Thailand, with no additional efficacy demonstration required.

The HAV vaccine study had a direct bearing on a study of the efficacy of a baculovirus-expressed recombinant HEV vaccine by reinforcing the need for a case definition that included clear evidence of liver injury, virus detection by PCR, multiple specimens collected early in disease, and longer follow up of cases. Conducted in 2000 Nepalese soldiers by an international team of investigators at another AFRIMS laboratory, known as the Walter Reed Army Research Unit-Nepal (WARUN), the study demonstrated that the efficacy of the HEV vaccine was 95% [121,122]. Although the hepatitis E vaccine proved to be highly efficacious, the manufacturer of this particular candidate has not further developed it, in part because of uncertainties regarding the public health priority for hepatitis E prevention and control through immunization.

The trials of JEV and HAV vaccines together set examples for the large-scale HIV vaccine efficacy trial conducted later as a Thai-US collaborative effort [123]. Expanded dengue vaccine trials, now being conducted in KPP, will benefit from the experiences obtained in earlier trials in that province.

4.2.3. Medical benefits

As a result of studies conducted in KPP, a number of specific products became available for much larger populations. In Thailand, studies suggested that the JEV vaccine would be cost effective [124,125] and vaccine usage was systematically expanded to progressively greater numbers of provinces, followed by a decline in cases reported, suggesting that thousands of cases have been prevented by implementation of JE immunization.

4.2.4. Community benefits

Nearly 200,000 residents of KPP volunteered for the studies that have been conducted in their province. Informal follow up

Table 2
Challenges in conducting field studies and the lessons learned from the m.

Positive/negative aspects of trials	Lesson learned
Japanese encephalitis efficacy trial	
Initially, relationships between agencies (AFRIMS, MOPH, KPP Hospital, and BIKEN) were poorly defined. Investigator provided all parties with a weekly written update	Communication by investigator team and concerned parties may help cement relationships
Prior to ICH Guidelines, investigators improvised many solutions	ICH guidelines provide useful checklists for investigators
A serious adverse event in a study participant led investigators to consider terminating the study. Following investigation, the MOPH Oversight Board recommended that the trial continue	An oversight board, sometimes called a Data and Safety Monitoring Board, can provide important guidance
Efficacy trial results were published, but impact in Thailand required manufacture and introduction of the vaccine and in the US, the FDA required one additional study for licensure	"Excellence in Research is Not Enough": Research must be followed by regulatory and logistical activities and vaccine distribution
Hepatitis A	
Invention and testing of the vaccine required interactions of WRAIR, NIAID, AFRIMS, MOPH, KPP, and a manufacturer	Relationships are essential elements of success in complex international endeavors
Based on unfavorable cost benefit studies, HAV vaccine was not introduced into the Thai EPI, though it was used for outbreak suppression and other focal uses	Re-analysis of costs and benefits may suggest suitable remedies
US FDA unexpectedly requested demonstration of etiologic agent in stool specimens from cases	Robust case definitions, with demonstration of etiological agents, should be agreed to at outset
Dengue	
Quality control of clinical observations, such as temperature, blood pressure, spleen size determinations, presence of petechia, and of serological testing may require standardization, training, and validation as well as photographic documentation. Methods for "routine tests" like virus neutralization tests, may vary greatly from lab to lab	Care must be exercised that all participating staff are trained to obtain data in a uniform and reproducible way and that all measurements are validated before beginning phase three trials
Many competing vaccines, rising cost of studies, and commercial interests that may differ from public health considerations complicate the selection of candidate vaccines and sites	Strive for common diagnostic tests and comparative (head to head) trials with predefined endpoints whenever possible
Influenza	
Adding new tests and procedures presented challenges in standardization and quality control	Special training for all procedures should be sought at global centers of excellence

conversations, provided by one of the authors (BLI), suggest that the organized clinical trials, with the informed consent processes that were mandated by the Institutional Review Boards resulted in a better-informed and medically aware citizenry in the province [126].

4.2.5. Challenges and positive/negative aspects of the conduct of the trials and lessons learned from conducting these studies (Table 2)

Studies that involve scores of investigators from different countries, multiple governmental Institutional Review Boards (IRBs), and hundreds of thousands of subjects are filled with complexity. Millions of data points may converge to answer a single question: Is the vaccine efficacious or not? With such complexity, there is room for learning many lessons, many of which are now incorporated into the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines for Good Clinical Practices, a document that was not available in 1982 [127] (Table 2).

In addition, several overarching principals are related to the conduct of studies with investigators of different nations, governed by different rules, working in different languages with parents and subjects who recognize the medical problem from its effects on their community. To identify research needs, potential investigators must be attuned to the advice of mentors and consultants. Where multiple vaccine candidates exist, head-to-head trials are desirable, but often resisted by manufacturers, so standardization and validation of case definitions and serological tests may suffice to facilitate informative comparisons.

Second, complex studies require a great deal of trust between individuals and between organizations, which must ultimately work as a team. Teams are said to move through several stages, including "Forming, Storming, Norming, and Performing" as they mature [128]. Bringing together IRB reviewers, oversight committees, vaccine manufacturers, study populations, collaborating investigators, and scores of supporting staff members is, in effect, an exercise in team building. Teams working in KPP followed this pattern, initially finding their way through occasionally turbulent waters, eventually developing trusting relationships, with many years of performing successful studies. The founding of KAVRU represents a physical manifestation of team building over the decades of collaboration before and after its founding.

Third, the subtle details related to the collection of clinical observations, and analysis of specimens must be carefully managed to prevent unintentional degradation of study results. Careful training and validation of all measurements and assays is essential.

Finally, elegant study design and compelling results are of little worth if the findings do not result in meaningful implementation. Following the JEV vaccine and HAV vaccine efficacy trials, both vaccines were advanced substantially toward licensure, and the results of the two studies carried out in KPP are mentioned prominently in the package inserts. Both vaccines became available for use in US citizens. HAV cases have fallen dramatically in the US since the introduction of the vaccine. In Thailand, the MOPH determined that the JEV vaccine would be cost effective, manufacturing facilities were constructed, and the vaccine added to routine immunizations with a reduction in the numbers of encephalitis cases. The hepatitis A vaccine was not introduced as a routine immunization in Thailand, a result that may have been related to the relative mildness of hepatitis A infections in young people. Perhaps conclusions will be altered if hepatitis surveillance should find a growing burden of disease of if vaccine cost should fall.

4.3. Future studies

The success of the studies of JEV and HAV and the initiation of the ongoing dengue vaccine study, combined with the characterization of the province's disease burden, suggest that additional future studies, perhaps of dengue and influenza, done at KAVRU may yield valuable information and products for treatment or prevention of these illnesses.

Table 3
Acknowledgment of contributions.

Participant	Role
Albert Sabin	Led effort to make JEV Vaccine during WWII
Richard Mason	Member of founding delegation from WRAIR
Kenneth Goodner	Provided introduction to Thai Classmate from Jefferson Medical College
Luang Binbakya Bidybhed	As Thai Undersecretary of State for Health, arranged initial meeting with King of Thailand. First Director General of SEATO Cholera Research Laboratory
Franklin Top	Established filter paper method of transporting dried sera to lab for testing for dengue antibody
Phillip Russell	As investigator at AFRIMS, co-developed dengue neutralizing antibody assay. As Commander, USAMRMC, supported concept of Japanese encephalitis vaccine efficacy trial
William Bancroft	Conducted studies of hepatitis A and dengue at AFRIMS and provided leadership for HAVV at WRAIR
Robert McNair Scott	Performed epidemiological studies of dengue. Led HEVV study in Nepal
Michael Benenson, Frank Sodetz	Commanders, USA Medical Component, AFRIMS during JEV studies
Ananda Nisalak	AFRIMS' senior Thai investigator, known and respected throughout Thailand. Co-Inventor of JE MAC ELISA test. Suggested test site in KPP. Coordinated large JE vaccine efficacy trial. Provided
Donald Burke	Invented test for Japanese encephalitis and supported JEVV trial Launched (with Dr Ananda) the first AFRIMS studies in KPP Oversaw invention of inactivated hepatitis A vaccine
Michael Ussery	AFRIMS co-investigator of test for Japanese encephalitis
Thanom Laorakpongse	KPP Hospital Director, senior collaborator
Nursing and Lab Staff	At KPP Hospital Provided patient care and participated in studies
Nursing and Lab Staff	At AFRIMS, provided clinical data collection for clinical and epidemiological studies and vaccine trials. Provided laboratory test development and support
Charles Hoke	At AFRIMS, formulated and led JE vaccine efficacy trial and dexamethasone treatment study, at WRAIR, chaired hepatitis and flavivirus vaccine steering committees and at USAMRMC, directed Military Infectious Diseases Research Program
Somnuek Lamjiak	Provided nursing support, unacknowledged in JEV vaccine trial paper
Suchard Jetanesen	At Thai MOPH CDC, conducted disease surveillance for Thailand that identified location of annual encephalitis outbreaks
Natth Bhamaraphavati	Distinguished Thai physician/scientist who suggested link between AFRIMS and Thai NIH
Nathirat Sangawhipa	At Thai NIH, identified need for JE Vaccine efficacy trial and supported proposed trial
Konosuke Fukai	At BIKEN Foundation, manufactured JEVV used in efficacy trial
Walter Brandt	At USAMMDA, convened meeting at USFDA, including vaccine manufacturer and US distributor, to review JEVV efficacy data and discuss additional data required for licensure in the US
Mary Kaye Gentry, Erik Henchal, Walter Brandt, Joel Dalrymple	At WRAIR, Identified and evaluated monoclonal antibodies for identifying and typing dengue and Japanese encephalitis and other flaviviruses
Edmund Tramont	Supported protocol for JE Vaccine Efficacy Trial
Stanley Lemon	Developed tests and laid groundwork for HAVV development
Leonard Binn	At WRAIR, invented first HAVV and showed protection of monkeys. Developed neutralizing antibody test
Kenneth Eckels	At WRAIR, manufactured first GMP batch of hepatitis A vaccine
Maria Sjogren	Led first clinical trial of WRAIR's hepatitis A vaccine
John Boslego	AFRIMS commander during hepatitis A vaccine efficacy trial
Bruce Innis	At AFRIMS, supported JEVV trial, advanced study of ELISA test for JE and dengue, conducted studies of HAV, proposed and led HAVV trial, proposed hepatitis E vaccine efficacy trial in Nepal, and organized team at WRAIR to invent replacement JEV vaccine produced in vero cells
Rapin Snitbahn	AFRIMS' senior Thai hepatitis investigator
Prayura Kunasol	As head of Thai CDC, advocated for HAV vaccine trial
Erich D'Hondt	Coordinated HAVV technology transfer from WRAIR to SKB
David Krause	At SKB, coordinated clinical development of HAV vaccine
Suchitra Nimmannitya, Siripen Kalayanarooj	Established grading of DHF and clinical treatment. Provided expert consultation on management of dengue to KPP hospital staff
Frank Ennis, Alan Rothman, Sharone Green	At University of Massachusetts, conducted numerous US NIH funded studies of dengue pathogenesis
Supamit Chunsuttiwat	Thai MOPH spokesperson for Thai Immunization Program that adopted JE vaccine. Co-investigator on dengue pathogenesis studies
David Vaughn	Led numerous studies of dengue immunology and pathogenesis, validating procedures. Secured funding for KAVRU construction. Directed Military Infectious Diseases Research Program
Mammen Mammen	Led dengue school-based and village cluster studies in KPP and supported HEV vaccine trial in Nepal. Designed and executed the construction of KAVRU
James W. Jones, Tom W. Scott	From AFRIMS and the University of California, Davis: Provided entomological studies following startup of KAVRU. Led the entomology contributions to KPSII cluster investigations
Timothy Endy	Conducted numerous school-based studies of dengue epidemiology in KPP and initiated the HEV trial in Nepal
Stephen Thomas	Studied dengue epidemiology and led WRAIR's dengue vaccine effort
Kamchai Rungsimunpaiboon	KPP Hospital Director
Tawee Chotpitayasunondh	As consultant, Thailand Ministry of Public Health, served as country coordinating investigator for dengue vaccine trial
Punnee Pitisuttithum	At Faculty of Tropical Medicine, Mahidol University, served as KPP Site Principal Investigator, dengue vaccine efficacy trial
Robert Gibbons	Led studies of dengue, Japanese encephalitis and influenza Conceived of the idea for this paper
In-Kyu Yoon	At AFRIMS, led virology department during dengue vaccine trials
Chusak Pimgate, Darunee Tannitisupawong	KAVRU site Directors since the opening of KAVRU
Chunlin Zhang	Established PCR capability at KAVRU in support of dengue cluster studies

Table 4
Acronyms used.

Name	Acronym
Armed Forces Research Institute of Medical Sciences	AFRIMS
Center for Disease Control	CDC
Cerebrospinal Fluid	CSF
Dengue Hemorrhagic Fever	DHF
Dengue Shock Syndrome	DSS
Enzyme Linked Immunosorbent Assay	ELISA
Expanded Program on Immunizations	EPI
Good Clinical Practices	GCP
Good Manufacturing Practices	GMP
GlaxoSmithKline	GSK
Hepatitis A virus	HAV
Hepatitis E virus	HEV
Human Immunodeficiency virus	HIV
Human Leukocyte Antigen	HLA
Immunoglobulin G	IgG
Immunoglobulin M	IgM
Immunoglobulin G antibody capture	GAC
Immunoglobulin M antibody capture	MAC
Institutional Review Board	IRB
International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use	ICH
Japanese encephalitis virus	JEV
Japan International Cooperation Agency	JICA
Kamphaeng Phet Province	KPP
Kamphaeng Phet-AFRIMS Virology Research Unit	KAVRU
Ministry of Public Health	MOPH
National Institutes of Health	NIH
Reverse Transcriptase Polymerase Chain Reaction	RT-PCR
Ribonucleic Acid	RNA
SEATO Medical Research Laboratory	SMRL
SmithKline Beecham	SKB
South East Asia Treaty Organization	SEATO
United Nations Educational, Scientific, and Cultural Organization	UNESCO
United States Food and Drug Administration	USFDA
US Army Medical Materiel Development Activity	USAMMDA
US Army Medical Research and Materiel Command	USAMRMC
Walter Reed Army Institute of Research	WRAIR
Walter Reed Army Research Unit-Nepal	WARUN

5. Conclusions

The collaboration between the Thai MOPH, KPP, and AFRIMS has resulted in a productive relationship of 30 years, culminating in the establishment of the Kamphaeng Phet-AFRIMS Virology Research Unit (KAVRU) and in the publication of approximately 80 papers. There have been benefits to all partners: Participants in vaccine trials benefited directly by increased medical surveillance and/or by administration of a beneficial vaccine at the end of the trial. The children of Thailand benefited from the introduction of the JEV into the Expanded Program on Immunizations (EPI) and, to a lesser extent, from the targeted use of HAV vaccine. The local community received training and mentorship and improved facilities for diagnosis of JE, hepatitis, dengue, and influenza. Investigators participated in valuable studies of the efficacy of JEV vaccine and HAV vaccine, paving the way for ongoing studies of dengue vaccines and studies of HIV and HEV vaccines. The Thai MOPH furthered its mission by conducting encephalitis surveillance that illuminated a problem so that the need for vaccine could be perceived. The vaccine manufacturers received information that supported applications for regulatory approval and, in the case of JE, allowed Japan to provide assistance in improving Thai vaccine capabilities. The US Army was able to identify, test, and procure two vaccines to protect service members against longstanding threats to their health. The general population of the US obtained access to two products valuable to travelers. And, by demonstrating the efficacy of these two vaccines and the reduced incidence of both diseases following use of the vaccines, the global community received the benefit of a successful international collaboration contributing to the improvement of complex public health problems. The multitude of beneficial outcomes from basic research to applied public health is in keeping with the guidance provided by a former AFRIMS investigator, Commander of the WRAIR and of the Army Medical Research and Materiel Command and President of the American Society for Tropical Medicine and Hygiene that “Excellence in Research is Not Enough” [129] (Table 3).

The acronyms used in this study are listed in Table 4.

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Disclaimer

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[126] Communication provided by Dr. Bruce Innis "The last time I was in KPP was 2006. I went looking to purchase some wine for a dinner. The attendant in the store was a young adult, around 25 years of age. We chatted about why I was in KPP and I explained I was escorting colleagues who were considering the feasibility of a dengue vaccine study in KPP. She said she had been in a hepatitis vaccine project 15 years earlier and I explained that I knew something about that and moreover, because of that study, a new vaccine was licensed for use globally. She was pleased, but much more interested to meet one of the foreigners who helped initiate the trial in her youth and to know that perhaps there would be another field trial someday concerning dengue. I was struck by her recall - that hepatitis trial made an impression on her (and me too)! I suppose the same is true for participants in all of the major multi-year cohort studies involving children there.".
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